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*Institute Report No. 296*

**Mutagenic Potential of Triethyleneglycol Dinitrate  
in the Ames *Salmonella*/Mammalian  
Microsome Mutagenicity Test**

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and  
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**GENETIC TOXICOLOGY BRANCH  
DIVISION OF TOXICOLOGY**

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**September 1988**

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*for Richard A. Kishimoto*  
\_\_\_\_\_  
Edwin S. Beatrice

COL, MC  
Commanding

*28 Sep 88*  
\_\_\_\_\_  
(date)

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## ABSTRACT

The mutagenic potential of TRIETHYLENEGLYCOL DINITRATE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5  $\mu$ l/plate to 0.0016  $\mu$ l/plate in both the presence and absence of metabolic activation. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, TRIETHYLENEGLYCOL DINITRATE, TEGDN, Propellant



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Letterman Army Institute of Research  
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US Army Medical Research and Development Command  
US Army Biomedical Research and Development Laboratory  
Fort Detrick, MD 21701-5010  
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLBO

GLP STUDY NUMBER: 84048

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD, MSC

PRINCIPAL INVESTIGATOR: Steven K. Sano, BA, SGT, USA

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOPs, stability, and purity data of the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: TRIETHYLENEGLYCOL DINITRATE (TEGDN)

INCLUSIVE STUDY DATES: 7-25 January 1985

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of TRIETHYLENEGLYCOL DINITRATE (LAIR Code TA044) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

## **ACKNOWLEDGMENTS**

CPT John W. Harbell, PhD, MSC, and Mr. John Dacey provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE  
STUDY

We, the undersigned, declare that GLP study number 84048 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr. 28 July 88  
DON W. KORTE JR, PhD / DATE  
MAJ, MSC  
Study Director

Conrad Wheeler 19 July 88  
CONRAD WHEELER, PhD / DATE  
DAC  
Analytical chemist

Steven K. Sang 5 MAR 86  
STEVEN K. SANG, BA / DATE  
SGT, USA  
Principal Investigator



DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH  
PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129-6800

REPLY TO  
ATTENTION OF

SGRD-ULZ-QA

22 September 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance Statement

1. This is to certify that the protocol for GLP Study 84048 was reviewed on 4 December 1984.

2. The institute report entitled "Mutagenic Potential of Triethyleneglycol Dinitrate in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 113, was audited on 12 May 1987.

*Carolyn M. Lewis*  
CAROLYN M. LEWIS  
Chief, Quality Assurance



## TABLE OF CONTENTS

	PAGE
Abstract .....	i
Preface .....	iii
Acknowledgments .....	iv
Signatures of Principal Scientists .....	v
Report of the Quality Assurance Unit .....	vi
Table of Contents .....	vii
INTRODUCTION .....	1
Objective of the Study .....	1
MATERIALS AND METHODS .....	2
Test Compound .....	2
Test Solvent .....	2
Chemical Preparation .....	2
Test Strains .....	2
Test Format .....	3
Data Interpretation .....	4
Deviations from the Protocol/SOP .....	4
Storage of Raw Data and Final Report .....	4
RESULTS .....	5
DISCUSSION .....	5
CONCLUSION .....	10
REFERENCES .....	11

## TABLE OF CONTENTS

	PAGE
APPENDICES .....	12
APPENDIX A .....	13
APPENDIX B .....	15
OFFICIAL DISTRIBUTION LIST .....	20

**Mutagenic Potential of TRIETHYLENEGLYCOL DINITRATE in the Ames Salmonella/Mammalian Microsome Mutagenicity Test--Sano and Korte**

**INTRODUCTION**

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABRDL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USABRDL has tasked the Toxicology Branch, LAIR, to conduct an initial health effects evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity test, acute oral toxicity tests in rats and mice, acute dermal toxicity tests in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs. This report contains the results of a study to assess the mutagenic potential of TEGDN in the Ames Salmonella/Mammalian Microsome Mutagenicity Test.

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect those compounds which are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (2).

Objective of the Study

The objective of this study was to determine the mutagenic potential of TRIETHYLENEGLYCOL DINITRATE (LAIR Code TA044) by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test.

## **MATERIAL AND METHODS**

### Test Compound

Chemical Name: TRIETHYLENEGLYCOL DINITRATE

LAIR Code Number: TA044

Physical State: yellow oil

Source: Naval Ordnance Station  
Indian Head, MD

Storage: TRIETHYLENEGLYCOL DINITRATE was received and assigned the LAIR Code number TA044. The test compound was stored at room temperature (21°C) until use.

Chemical Properties/Analysis: Data characterizing the chemical composition and purity of the test material are presented in Appendix A.

### Test Solvent

The test compound and the positive control chemicals were dissolved in grade 1 dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO).

### Chemical Preparation

On the day before dosing, 500  $\mu$ l of the test compound was measured into a sterile vial and again stored at room temperature. On the day of dosing, the 500- $\mu$ l sample was dissolved in 9.4 ml of grade I dimethyl sulfoxide to achieve a 5% (v/v) solution. Aliquots of this solution were used to dose the test plates.

### Test Strains

*Salmonella* strains TA98, TA100, TA1535, TA1537, and TA1538, obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory at -80°C. Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (3).

Test Format

TRIETHYLENEGLYCOL DINITRATE was evaluated for mutagenic potential according to the methods of Ames et al (4). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (3).

**Toxicity Tests:** Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of TRIETHYLENEGLYCOL DINITRATE ranging from  $1.6 \times 10^{-3}$   $\mu$ l/plate to 5  $\mu$ l/plate and approximately  $10^8$  cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin was placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate.

**Mutagenicity Test:** The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5, both with and without 0.5 ml of the S-9 microsome fraction. The S-9 was purchased from Litton Bionetics (Kensington, MD). The optimal titer of this S-9, as determined by Litton Bionetics, was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" concentrate (5). The water used in this medium and in all reagents came from a Polymetric model 200-3 Water Purifier (Sunnyvale, CA). Plates were incubated upside down in the dark, at 37°C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Ames et al (4). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The integrity

Sano and Korte--4

of the different *Salmonella* strains used in the assay was verified by the following standard tests:

-Lack of growth (inhibition) in the presence of crystal violet which indicates that the prerequisite alteration of the lipopolysaccharide layer of the cell wall is present.

-Growth in the presence of ampicillin-impregnated disks which indicates the presence of an ampicillin-resistant R Factor in the TA98 and TA100 strains.

-Lack of growth (inhibition) following exposure to ultraviolet light indicates the absence of the DNA excision-repair mechanism.

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control, but may be tested with several. These compounds (benzo[a]pyrene, 2-aminofluorene, 2-aminoanthracene and N-methyl-N'-nitro-N-nitrosoguanidine) were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and the known mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

#### Data Interpretation

According to Brusick (6), a compound is considered mutagenic if a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count for the tester strains TA98 and TA100, or three times the spontaneous colony count for strains TA1535, TA1537, and TA1538. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

#### Deviations from the Protocol/SOP

None.

#### Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR archives.

## RESULTS

On 16 January 1985, the toxicity of TRIETHYLENEGLYCOL DINITRATE was determined (Table 1). For this experiment all sterility, strain verification, and negative controls were normal (Table 1). Exposure of the tester strain (TA100) to the highest dose showed neither a decrease in the number of macrocolonies nor an observable reduction in the density of the background lawn, indicating no chemical toxicity. Therefore, the highest dose selected for the mutagenicity test was 5  $\mu$ l/plate.

Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 23-25 January 1985 (Table 2). TRIETHYLENEGLYCOL DINITRATE did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3).

A copy of the raw data is included in Appendix B.

## DISCUSSION

Certain test criteria must be satisfied before the Ames Test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alteration in the LP (lipopolysaccharide) layer, and deficiency in DNA excision-repair. Second, the *Salmonella* strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of the Ames test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, TRIETHYLENEGLYCOL DINITRATE was evaluated in the Ames Test. Criteria for a positive response include both a correlated dose-response over three dose concentrations and a revertant colony count at least two times (TA98, TA100) or three times (TA1535, TA1537, TA1538) the spontaneous revertant colony count (6). TRIETHYLENEGLYCOL DINITRATE did not induce the requisite dose response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that TRIETHYLENEGLYCOL DINITRATE is not mutagenic when evaluated in the Ames Test.

Sano and Korte--6

**TABLE 1: TOXICITY DETERMINATION FOR TEGDN (TA044)**

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GLP STUDY NUMBER: 84048      16 JAN 85      PERFORMED BY: SANO

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**TA 100 REVERTANT PLATE COUNT**

TEST COMPOUND CONCENTRATION	MEAN (1SD)	BACKGROUND LAWN*
NEGATIVE CONTROL	95 (4.6)	NR
5.0 µl/plate	99 (13.4)	NL
1.0 µl/plate	96 (17.2)	NL
0.2 µl/plate	107 (17.6)	NL
0.04 µl/plate	85 (8.3)	NL
0.008 µl/plate	110 (2.1)	NL
0.0016 µl/plate	97 (6.0)	NL

**STRAIN VERIFICATION FOR TOXICITY DETERMINATION**

TA 100\*

HISTIDINE REQUIREMENT	NG
AMPICILLIN RESISTANCE	G
UV	NG
CRYSTAL VIOLET SENSITIVITY	NG (13mm)
STERILITY CONTROL	NG

**STERILITY CONTROL FOR TOXICITY DETERMINATION**

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

---

\* NL=Normal Lawn, G=Growth, NG=No Growth, ST=Slight Toxicity  
NR=Not Recorded



**TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING  
FOR THE MUTAGENICITY DETERMINATION OF TEGDN (TA044)**

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GLP STUDY NUMBER: 84048    7-25 JAN 85    PERFORMED BY: SANO

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**STRAIN VERIFICATION**

**OBSERVATIONS\***

STRAIN	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET (zone size)	CONTROL
TA98	NG	G	NG	NG (14mm)	NG
TA100	NG	G	NG	NG (10mm)	NG
TA1535	NG	NG	NG	NG (12mm)	NG
TA1537	NG	NG	NG	NG (13mm)	NG
TA1538	NG	NG	NG	NG (10mm)	NG

**STERILITY CONTROL FOR MUTAGENICITY DETERMINATION**

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

---

\* NL=Normal Lawn, G=Growth, NG=No Growth, ST=Slight Toxicity

TABLE 3: MUTAGENICITY ASSAY FOR TEGDN (TA044)

REVERTANTS/PLATE (MEAN  $\pm$ 1SD)

COMPOUND	DOSE/PLATE	TA98		TA100	
		WITHOUT S-9		WITH S-9	
START NEG CONTROL	0.0 $\mu$ g	16	$\pm$ 4.0	88	$\pm$ 10.2
MNNG†	2.0 $\mu$ g	-	-	1017	$\pm$ 167.8
MNNG†	20.0 $\mu$ g	-	-	-	-
TA044	5 $\mu$ l	14	$\pm$ 6.6	42	$\pm$ 10.2
TA044	1 $\mu$ l	24	$\pm$ 5.6	78	$\pm$ 5.5
TA044	0.2 $\mu$ l	20	$\pm$ 4.7	109	$\pm$ 3.8
TA044	0.04 $\mu$ l	22	$\pm$ 4.7	76	$\pm$ 12.5
TA044	0.008 $\mu$ l	27	$\pm$ 7.0	84	$\pm$ 11.3
TA044	0.0016 $\mu$ l	24	$\pm$ 3.6	95	$\pm$ 20.2
END NEG CONTROL	0.0 $\mu$ g	15	$\pm$ 5.0	108	$\pm$ 7.9
START NEG CONTROL	0.0 $\mu$ g	27	$\pm$ 2.1	86	$\pm$ 15.1
2-AA†	2.0 $\mu$ g	976	$\pm$ 366.9	1635	$\pm$ 30.4
2-AF†	2.0 $\mu$ g	444	$\pm$ 135.5	533	$\pm$ 144.6
BP†	2.0 $\mu$ g	180	$\pm$ 29.5	509	$\pm$ 19.7
TA044	5 $\mu$ l	23	$\pm$ 1.5	95	$\pm$ 14.0
TA044	1 $\mu$ l	20	$\pm$ 4.0	88	$\pm$ 20.5
TA044	0.2 $\mu$ l	24	$\pm$ 3.6	72	$\pm$ 8.4
TA044	0.04 $\mu$ l	21	$\pm$ 1.5	57	$\pm$ 11.6
TA044	0.008 $\mu$ l	26	$\pm$ 7.0	101	$\pm$ 30.0
TA044	0.0016 $\mu$ l	27	$\pm$ 2.6	121	$\pm$ 14.8
END NEG CONTROL	0.0 $\mu$ g	24	$\pm$ 3.5	106	$\pm$ 8.0

† MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; 2-AA = 2-aminoanthracene; 2-AF = 2-aminofluorene; BP = benzo[a]pyrene.

TABLE 3 (cont.): MUTAGENICITY ASSAY FOR TEGDN (TA044)

REVERTANTS/PLATE (MEAN  $\pm$ 1SD)

COMPOUND	DOSE/PLATE	TA1535	TA1537	TA1538
<b>WITHOUT S-9</b>				
START NEG CONTROL	0.0 $\mu$ g	13 $\pm$ 1.5	8 $\pm$ 2.0	23 $\pm$ 3.6
MNNG†	2.0 $\mu$ g	-	-	-
MNNG†	20.0 $\mu$ g	1812 $\pm$ 217.2	-	-
TA044	5 $\mu$ l	23 $\pm$ 4.9	5 $\pm$ 0.6	30 $\pm$ 4.6
TA044	1 $\mu$ l	18 $\pm$ 4.2	4 $\pm$ 3.8	31 $\pm$ 10.4
TA044	0.2 $\mu$ l	21 $\pm$ 3.5	4 $\pm$ 2.3	29 $\pm$ 3.0
TA044	0.04 $\mu$ l	25 $\pm$ 2.3	8 $\pm$ 0.6	44 $\pm$ 2.0
TA044	0.008 $\mu$ l	19 $\pm$ 2.6	4 $\pm$ 1.5	35 $\pm$ 6.0
TA044	0.0016 $\mu$ l	17 $\pm$ 5.8	5 $\pm$ 2.1	35 $\pm$ 5.7
END NEG CONTROL	0.0 $\mu$ g	21 $\pm$ 2.3	6 $\pm$ 1.0	29 $\pm$ 5.9
<b>WITH S-9</b>				
START NEG CONTROL	0.0 $\mu$ g	13 $\pm$ 2.5	6 $\pm$ 3.8	28 $\pm$ 4.0
2-AA†	2.0 $\mu$ g	-	245 $\pm$ 56.5	1286 $\pm$ 102.0
2-AF†	2.0 $\mu$ g	-	-	559 $\pm$ 82.5
BP†	2.0 $\mu$ g	-	44 $\pm$ 3.6	88 $\pm$ 4.0
TA044	5 $\mu$ l	21 $\pm$ 4.6	6 $\pm$ 1.2	25 $\pm$ 5.0
TA044	1 $\mu$ l	22 $\pm$ 3.1	7 $\pm$ 1.5	19 $\pm$ 8.0
TA044	0.2 $\mu$ l	17 $\pm$ 3.1	4 $\pm$ 3.5	30 $\pm$ 3.6
TA044	0.04 $\mu$ l	12 $\pm$ 3.1	6 $\pm$ 1.2	19 $\pm$ 7.0
TA044	0.008 $\mu$ l	17 $\pm$ 4.2	4 $\pm$ 2.1	27 $\pm$ 6.2
TA044	0.0016 $\mu$ l	18 $\pm$ 4.4	5 $\pm$ 1.2	30 $\pm$ 1.2
END NEG CONTROL	0.0 $\mu$ g	14 $\pm$ 1.7	4 $\pm$ 1.0	25 $\pm$ 4.0

† MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; 2-AA = 2-aminoanthracene; 2-AF = 2-aminofluorene; BP = benzo[a]pyrene.

Sano and Korte--10

### **CONCLUSION**

TRIETHYLENEGLYCOL DINITRATE was evaluated for mutagenic potential in the Ames Test, in both the presence and absence of metabolic activation, and did not produce a positive mutagenic response under conditions of this study.

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1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846.
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6. Brusick D. Genetic Toxicology. In: Hayes AW, ed. Principles and Methods of Toxicology. New York: Raven Press, 1982: 223-272.

Sano and Korte--12

Appendix A. Chemical Data.....	13
Appendix B. Individual Plate Scores.....	15

**APPENDIX A: CHEMICAL DATA**

Chemical Name: Ethanol, 2,2'-[1,2-ethanediylbis(oxy)] bis-,  
dinitrate

Alternate Chemical Names: Triethyleneglycol dinitrate  
(TEGDN), NOSET-A

Chemical Abstracts Service Registry No.: 111-22-8

LAIR Code No.: TA044

Chemical Structure:



Molecular Formula:  $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_8$

Molecular Weight: 240

Physical State: Yellow oil

Density: ( $\text{g}/\text{cm}^3$ ): 1.32\*

Source: Naval Ordnance Station, Indian Head, MD, 20640

Lot No.: 130-84

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\* Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846, p17.

**APPENDIX A (cont.): CHEMICAL DATA**

Analytical data: The compound chromatographed as a single peak (retention time 5.8 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% methanol; flow rate 0.9 ml/min, detection wavelength, 215 nm.<sup>†</sup> No impurities were detectable by NMR.<sup>‡</sup> NMR (80 MHz, CDCl<sub>3</sub>): 3.65 (s, 4H, -CH<sub>2</sub>-O-CH<sub>2</sub>CH<sub>2</sub>-O-CH<sub>2</sub>-), 3.72-3.84 (Complex multiplet, 4H, terminal methylene groups). IR (KBr): 2900, 1630, 1280, 1130, 1030, 910, 860 cm.<sup>§</sup>

Stability: The compound was received as a 10% solution in ethanol. Periodic analysis of this solution by HPLC has shown no evidence of decomposition to date (4 months).<sup>†</sup> NMR analysis demonstrated that the neat compound is stable for at least 1 month.<sup>‡</sup>

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<sup>†</sup> Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p26-30, 42-43. Letterman Army Institute of Research, Presidio of San Francisco, CA.

<sup>‡</sup> Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p63. Letterman Army Institute of Research, Presidio of San Francisco, CA.

<sup>§</sup> Ibid. p64.



## APPENDIX B: INDIVIDUAL PLATE SCORES

TRIETHYLENEGLYCOL DINITRATE (TA044)  
TOXICITY TEST  
TA100

DOSES	5.0 $\mu$ l/p	1.0 $\mu$ l/p	0.2 $\mu$ l/p	0.04 $\mu$ l/p
PLATE 1	114	116	87	78
PLATE 2	89	84	114	94
PLATE 3	93	89	120	82
background lawn	normal lawn	normal lawn	normal lawn	normal lawn

  

DOSES	0.008 $\mu$ l/p	0.0016 $\mu$ l/p	NEG CONTROL
PLATE 1	111	97	90
PLATE 2	112	91	98
PLATE 3	108	103	98
background lawn	normal lawn	normal lawn	not recorded

**APPENDIX B (cont.): INDIVIDUAL PLATE SCORES**NEGATIVE CONTROLS FOR MUTAGENICITY TESTS  
TRIETHYLENEGLYCOL DINITRATE (TA044)

COMPOUND	DOSE	TA98	TA100	TA1535	TA1537	TA1538
<u>WITHOUT S-2</u>						
NEG CONTROL (START RUN)	0.0 µg/plate	20 12 16	91 77 97	15 12 13	8 10 6	20 27 22
NEG CONTROL (END RUN)	0.0 µg/plate	10 20 14	99 114 111	20 20 24	7 5 6	22 31 33
<u>WITH S-2</u>						
NEG CONTROL (START RUN)	0.0 µg/plate	28 25 29	74 81 103	13 11 16	2 9 8	29 32 24
NEG CONTROL (END RUN)	0.0 µg/plate	20 27 24	98 114 106	12 15 15	4 5 3	21 24 29

## APPENDIX B (cont.): INDIVIDUAL PLATE SCORES

POSITIVE CONTROLS FOR MUTAGENICITY ASSAY  
TRIETHYLENEGLYCOL DINITRATE (TA044)

COMPOUND†	DOSE	TA98	TA100	TA1535	TA1537	TA1538
2-AA	2.0 µg/plate	1352 619 956	1600 1647 1657		288 181 266	1283 1185 1389
2-AF	2.0 µg/plate	370 600 361	369 586 643			600 613 464
BP	2.0 µg/plate	206 148 186	500 532 496		41 48 43	93 86 86
MNNG	2.0 µg/plate		926 1211 915			
MNNG	20.0 µg/plate			2047 1619 1769		

† MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; 2-AA = 2-aminoanthracene; 2-AF = 2-aminofluorene; BP = benzo[a]pyrene.

**APPENDIX B (cont.): INDIVIDUAL PLATE SCORES**  
**TRIETHYLENEGLYCOL DINITRATE (TA044)**  
**MUTAGENICITY TESTS**

**WITHOUT S-9**

COMPOUND	DOSE	TA98	TA100	TA1535	TA1537	TA1538
TA044	5 µl/plate	15	38	20	5	26
		20	54	29	5	35
		7	35	21	4	29
TA044	1 µl/plate	23	74	21	2	38
		30	75	19	1	19
		19	84	13	8	36
TA044	0.2 µl/plate	18	113	18	3	29
		16	106	21	3	26
		25	107	25	7	32
TA044	0.04 µl/plate	20	70	26	8	44
		27	90	22	7	46
		18	67	26	8	42
TA044	0.008 µl/plate	20	91	20	6	36
		28	90	21	4	29
		34	71	16	3	41
TA044	0.0016 µl/plate	20	103	10	3	41
		25	72	20	7	30
		27	110	20	6	33

## APPENDIX B (cont.): INDIVIDUAL PLATE SCORES

TRIMETHYLOLETHANE TRINITRATE (TA044)  
MUTAGENICITY TESTS

## WITH S-9

COMPOUND	DOSE	TA98	TA100	TA1535	TA1537	TA1538
TA044	5 $\mu$ l/plate	25 23 22	105 79 101	26 17 20	7 5 5	30 20 26
TA044	1 $\mu$ l/plate	20 24 16	67 108 89	19 25 21	7 5 8	27 18 11
TA044	0.2 $\mu$ l/plate	21 28 23	62 77 76	14 20 18	8 2 2	26 31 33
TA044	0.04 $\mu$ l/plate	21 19 22	69 55 46	15 13 9	5 5 7	26 12 19
TA044	0.008 $\mu$ l/plate	33 25 19	77 108 117	12 18 20	5 6 2	20 32 29
TA044	0.0016 $\mu$ l/plate	25 26 30	128 131 104	20 21 13	6 4 6	31 31 29

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